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滇牡丹复合群的 Giemsa C-带比较研究

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摘要 应用 BSG 方法对滇牡丹（*Paeonia delavayi*）复合群 5 个类群的 Giemsa C-带进行了比较研究。在 5 个类群的根尖体细胞有丝分裂中期观察到 10 条染色体，其核型基本一致，均为 K＝2n＝10＝6m+2sm+2st。各类群的 10 条染色体都在着丝点附近显示出了 Giemsa C-带，所有染色体的长臂上都没有显示出 Giemsa C-带，而短臂上的 Giemsa C-带的数量和位置在类群之间表现了一定的差异。除了滇牡丹第一对同源染色体中只有一条的短臂上显示出了 Giemsa C-带而表现出了异染色质的杂合性外，其余各类群每一对同源染色体都显示了相同的 Giemsa C-带。每一类群所含的 C—带比率（C—带总长度／染色体总长度）非常接近，约为 10%。文中还对其形态性状进行了分析。作者认为滇牡丹复合群 5 个类群的关系非常密切，可能是由同一祖先演化而来的。

关键词 滇牡丹复合群，染色体，Giemsa C-带

C BANDING PATTERNS IN PAEONIA DELAVAYI COMPLEX OF GENUS PAEONIA

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Abstract The C-banding patterns of five taxa of *Paeonia delavayi* complex from Yunnan province in China were analyzed using a modified BSG technique. Each taxon (species or variety or form) examined had a unique C-banding pattern with some common features present in each taxon. All taxa examined were diploid with 2n＝10. Each taxon showed C-bands at the centromeric regions and no C-band at the long arms of all ten chromosomes. There were differences in the C-banding numbers and positions at the terminal regions of the short arms. The average ratio of C-band length to total chromosome length in each taxon was approximately 10% as found in *P. delavayi*. These results suggest that the five taxa of *P. delavayi* complex are closely related and may have a common ancestor.

Key words *Paeonia delavayi* complex, Chromosome, C-band

INTRODUCTION

Paeonia dalavayi complex, belonging to the genus *Paeonia*, Paeoniaceae, is retrictively distributed

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in southwest China. With regard to the complex taxonomical classification , confusions still remain. Sometimes, several scientific names are given to the same taxon by different researchers. The following is a brief introduction of the taxonomical hostory in *P. delavayi* complex.

Franchet(1886) described two new species of the genera *Paeonia, P. delavayi* Franch. and *P. lutea* Franch. ex Delavay, based on Delavay's specimen from Yunnan, China. Then, Finet and Gagnepain (1904) recognized *P. lutea* as a variety of *P. delavayi, P. delavayi* var. *lutea* (Franch. ex Delavay) Finet et Gagnep.. Rehder and Wilson (1913) described another variety of *P. delavayi* based on the Wilson's specimem from Yunnan, China, namely *P. delavayi* var. *angustiloba* Rehd. ex Wils.. Komarov (1921) described *P. potanini* Komarov according to Potany's specimem from Sichuan, China. *P. potanini* was *P. delavayi* var. *angustiloba.* Stapf (1931) published *P. trollioides* Stapf ex Stern, which was taxonomical treated as *P. potanini* var. *trollioides* (Stapf ex Stern) Stern by Stern (1943). Bean (1933) discovered *P. delavayi* var. *alba* Bean from cultivated plants introduced from Yunnan. Stern (1943) considered *P. delavayi* var. *alba* as *P. potanini* f. *alba* (Bean) Stern.

Based on the external morphological characteristics, such as flower colour, leaf shape and so on, different taxonomical revisions of *P. delavayi* complex were made by various authors. Stern (1946) originally divided the complex into three species with *P. potanini* having one variety and one form:

P. delavayi Franch.,

P. lutea Franch. ex Delavay,

P. potanini Komarov,

P. potanini has a variety and a form;

P. potanini var. *trollioides* (Stapf ex Stern) Stern,

P. potanini f. *alba* (Bean) Stern.

Fang Wenpei (1958) adopted Stern's taxonomical treatment in his study of the Genus *Paeonia* in China. However, Pan Kaiyu (1979) proposed another revision suggesting that the *P. delavayi* complex be treated as one species with two varieties:

P. delavayi Franch.,

P. delavayi var. *angustiloba* Rehd. et Wils.,

P. delavayi var. *lutea* (Franch. ex Delavay) Finet et Gagnep..

Karyomorphological studies on *P. delavayi* complex indicate a standard karyotype, 2n = 10 = 6m+2sm+2st (Li Sifeng *et al,* 1989; Yang Diqing *et al,* 1989; Gong Xun *et al,* 1991). Among the species and varieties in the *P. delavayi* complex, the large karyotypic components are relatively stable while the number of satellites and their positions on chromosomes are quite variable.

In this study, the C-banding patterns of *P. delavayi* complex were investigated using a modified BSG (barium hydroxide / saline Giemsa) method. The similarities and differences in the C-banding patterns in each of the types were studied for the first time.

MATERIALS AND METHODS

All the materials studied were collected from natural habitats and maintained at the Institute in a living collection with voucher specimens in the Herbarium, Kunming Institute of Botany . The plant material sources and identification numbers are shown in Table 1. Collectors assigned plant identification numbers

to samples collected in the field.

Table 1　Identification and locality of each *Paeonia* species in Yunnan, China

Taxon	Locality	Altitude (m)	Voucher
P. delavayi Franchet	Heibaishui, Zhongdian	2500	89-09
P. lutea Franchet ex Delavay	West Hill, Kunming	2100	88-03
P. potanini Komorov	Tuguangcun, Zhongdian	3200	89-03
P. potanini var. *trollioides* (Stapf ex Stern) Stern	Wenghui, Zhongdian	2960	89-07

The C-band data of the karyotypes were based on examination of at least 30 complete somatic cells from each taxon at metaphase obtained from at least ten root-tips of each type. The BSG C-banding technique reported by Tanaka and Taniguchi (1975) was modified and used as follows:

1. Actively growing root-tips were pretreated in 0.1% colchicine at 20 ℃ for 3 hrs.

2. The root-tips were fixed in a 95% ethanol and glacial acetic acid (3:1) mixture at 4 ℃ for 20 mins.

3. Specimens were hydrolyzed in the mixture of 1 N hydrochloric acid and 45% acetic acid at 60 ℃ for 30 seconds and rinsed in distilled water for 2 mins.

4. Meristematic cells of the root-tips were squashed in 45% acetic acid solution, and cover slips were removed after freezing with dry ice.

5. Single separated cells with visible chromosomes were air-dried for 48 hrs at 35 ℃ on slides.

6. Slides were incubated in 5% aqueous barium hydroxide solution at 45 ℃ for 5 mins and then rinsed in distilled water for 10 mins.

7. Each slide was incubated in 2 x SSC (0.3 M sodium chloride + 0.03 M tri-sodium citrate aqueous solution) at 60 ℃ for 2 hrs and then rinsed in distilled water for 10 mins.

8. Slides were dipped in 2% Giemsa-PBS solution (pH 6.8) for several minutes at room temperature and then thoroughly rinsed in distilled water.

9. Following air-drying, the specimens were cleared in xylol for 30 min and mounted permanently in Eukitt (Kindler, Germany).

The chromosomes were classified by arm ratio according to symbols used by Levan *et al.* (1964), and classification of C-banded chromosomes followed Taniguchi *et al.* (1975). Idiograms were drawn from the mean of ten somatic metaphases. The chromosomes were matched into pairs and the mean value for short and the long arms of each pair was ascertained. Chromosomes in each ideogram were arranged in order of decreasing lengths.

GENERAL DESCRIPTION

(1) *P. delavayi* Franchet (Plate Ⅰ: G-5; Fig.2: 5), has deep purple flowers, with bract and sepal numbers ranging from 8 to 14; the leaflobes are linear to lanceolate.

(2) *P. lutea* Franchet ex Delavay (Plate Ⅰ: C-3; Fig.2: 3), has yellow flowers with purple reddish spots on the base of petals, with bracts and sepals ranging from 5 to 9. The lobes of its leaf are similar to that of *P. delavayi* Franchet.

(3) *P. potanini* Komorov. (Plate Ⅰ: F-1; Fig.2: 1), was thought to be restricted to the western part of Sichuan province, China. However, in 1989, we found many plants of this species growing in Zhongdian,

Yunnan Province and successfully transferred some plants to Kunming Botanical Garden for further study. This species has orange reddish flowers, bract and sepal numbers similar to *P. lutea*, the leaflobes are more linear than that of *P. lutea* Franchet ex Delavay.

(4) *P. potanini* var. *trollioides* Stapf ex Stern (Plate Ⅰ: D–4; Fig.2: 4), has golden yellow flowers with a campanulate corolla. The bract and sepal numbers are similar to that of *P. lutea* Franchet et Delavayi and leaf lobes are the same as *P. potanini* Komorow. The scientific name, *P. potanini* var. *trollioides*, was treated as a synonym of *P. delavayi* var. *lutea* by Pan kaiyu (1979).

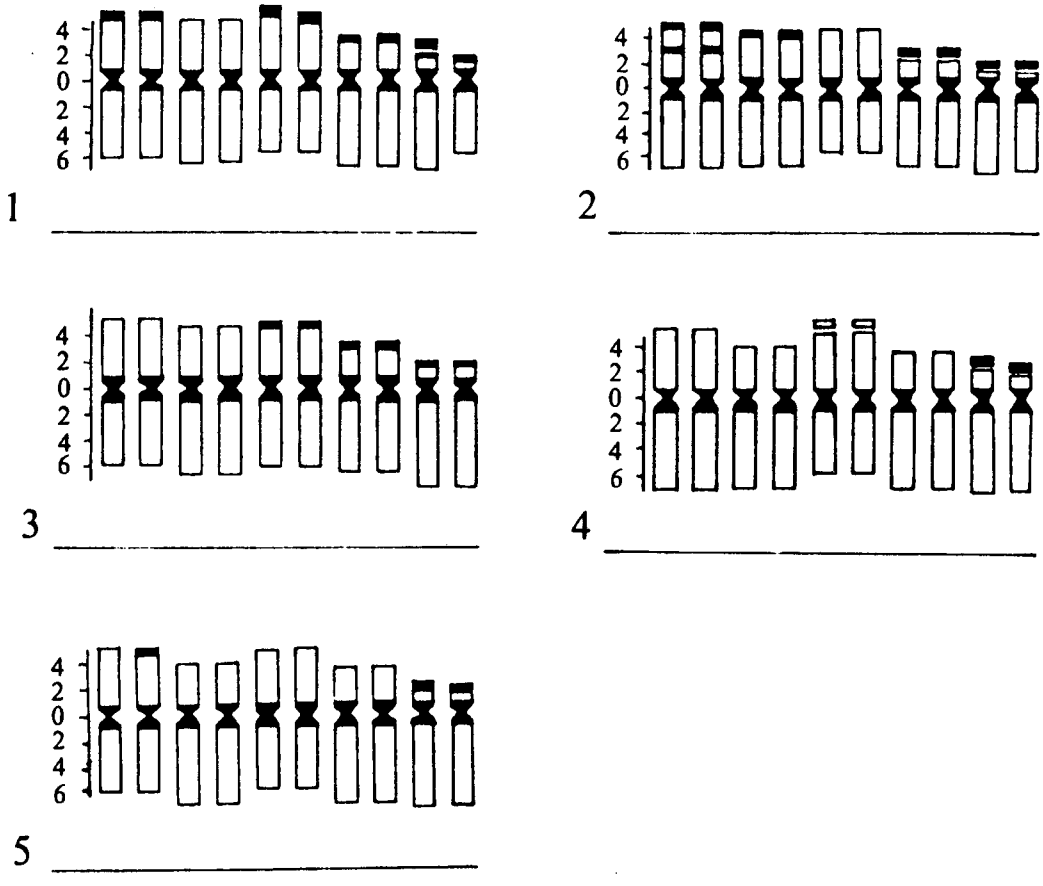

Fig. 1 Idiograms of C–band patterns of *P. delavayi* complex.

1. *P. potanini* Komorov; 2. *P. potanini* f. *alba* (Bean) Stern; 3. *P. lutea* Franchet ex Delavay; 4. *P. potanini* var. *trollioides* (Stapf ex Stern) Stern; 5. *P. delavayi* Franchet.

(5) *P. potanini* f. *alba* (Bean) Stern. (Plate Ⅰ: E–2; Fig.2: 2), was first described by Stern (1946) using cultivated plants without original locality. In 1989 we found this plant well grown on the west bank of the Mekong River in Weixi County, Yunnan Province, China. It is with white fragrant flowers whose numbers of bracts, and sepals and lobe shape of leaves are similar to that of *P. potanini* komorov.

RESULTS AND DISCUSSION

The C-banding metaphase, prophase and interphase chromosomes of five taxa examined are shown in Figures 1 and 2. All taxa examined were diploid with 2n = 10. The characteristics of the C-banding karyotypes are summarized in Table 2.

1. Interphase and prophase

About 10 rounds, granular chromocenters were darkly stained with the BSG technique and were observed at interphase nuclei(Plate Ⅰ: B). Up to prophase the C-bands appeared in the centromere region of each chromosome. The number of darkly stained regions in chromosomes of each type were stable.

2. Metaphase

The following results were observed:

(1) *P. delavayi* Franchet (Plate Ⅰ: G-5; Fig.2: 5).

The karyotype was 2n = 10 = 6m+2sm+2st. All ten chromosomes had C-bands in the centromeric regions. The second and fifth pairs of chromosomes had C-bands at the terminal regions of the short arms. The ratio of C-band length to total chromosome length was 10.0%.

(2) *P. lutea* Franchtet ex Delavay (Plate Ⅰ: C-3; Fig.2: 3). The karyotype is 2n = 10 = 6m+2sm+2st. All the chromosomes have C-bands at centromeric regions. The third, fourth and fifth pairs of chromosomes have, in addition, C-bands at the terminal regions. The ratio of C-band length to total chromosome length is 10.6%.

(3) *P. potanini* Komorov. (Plate Ⅰ: F-1; Fig.2: 1).

The karyotype was 2n = 10 = 6m+2sm+2st (1 satellite). All ten chromosomes showed C-bands at the centromeric regions. The first, third, fourth and fifth Pairs of chromosomes had C-bands at the terminal regions of the short arms. The ninth chromosome possessed a satellite on the short arm, which showed a C-band. The ratio of C-band length to total chromosome length was 12.5%.

Table 2　Numbers of chromosomes showing different C-banding patterns

Taxon	Karyotype (2n = 10)	C-Banding Pattern			Total length of C-band(%)
		OCO	DCO	DICO	
P. delavayi	6m+2sm+2st	8	2		10.0
P. lutea	6m+2sm+2st	6	4		10.6
P. potanini	6m+2sm+2st(1sat)	2	8		12.5
P. potanini var. *trollioides*	6m+2sm(2sat) +2st(2sat)	10			9.1
P. potanini f. *alba*	6m+2sm+2st	2	6	2	10.5

Note: OCO = C-band occupies the centromeric region of Chromosome;

CDO = C-band occupies the distal region of short arm and the centromeric region of chromosome.

DICO = C-band occupies the distal and interstitial region of short arm of chromosome, and the centromeric region of chromosome; Sat = satellite.

(4) *P. potanini* var. *trollioides* (Stapf ex Stern)Stern(Plate Ⅰ: D-4; Fig.2: 4).

The karyotype was 2n = 10 = 6m+2sm(2sat)+2st(2sat). All the ten chromosomes showed C-bands at the centromeric regions. The third and fifth pair chromosomes had satellites on the short arms, and the satellites on the fifth pair of chromosomes showed C-bands. The ratio of C-band length to total chromosome

length was 9.1%.

(5) *P. potanini* f. *alba* (Bean) Stern. (Plate Ⅰ: E-2; Fig.2: 2).

The karyotype was 2n=10=6m+2sm+2st. All the ten chromosomes showed C-bands at the centromeric regions. The first, second, fourth and fifth pairs of chromosomes showed C-bands at the terminal regions of the short arms, and the first pair of chromosome also showed thin and weak interstitial C-bands on the short arms. The ratio of C-band length to total chromosome length was 10.5%.

The karyotypes of *P. delavayi* complex have been studied by other investigators (Li Sifeng *et al*, 1989; Gong Xun *et al*, 1991; Yang Diqing *et al*, 1989). They state that the basic karyotype consists of six median, two submedians, and two subterminal centromeric chromosomes and within the taxon differ only in the number and position of satellites. The similarity of the karyotypic data presented in this paper confirms the earlier reports. Stebbins (1971) indicates that the karyotype for some plants including *Paeonia* exhibit a relatively constant asymmetry. Therefore, the karyotpye analysis alone would not permit discrimination of plants within the *P. delavayi* complex.

However, C-banding provides much more information about the karyotype architecture and reveals important differences in the distribution of herterochromatin. Each plant investigated here in this complex has a unique C-banding pattern. All types show C-bands at the certronmeric regions of all chromosomes, but each type has a characteristic C-banding at the terminal regions of the chromosomes, namely, the numbers and positions of C-bands at the terminal regions of chromosomes show the differences between types. But this difference has been seen between the populations of *P. lutea*. (The results will be reported elsewhere.) The ratio of C-band length to total chromosome length ranges from 9.1% (*P. potanini* var. *trollioides*) to 12.5% (*P. potanini*), but the average is near 10.1% which is the ratio in *P. delavayi*, a plant which is assumed to be the original species in the Sect. Moutan of Genus *Paeonia*. Thus, the C-banding patterns in this complex is not helpful to elucidate taxonomic relationships of this complex.

Analysis of the informative C-banding data using branch and bound parsimony with unordered characters reveals one potential unrooted parsimonious tree. While this tree does not indicate the order of evolution, the data do suggest two direct associations firstly between *P. potanini* and *P. potanini* f. *alba*, and secondly between *P. potanini* var. *trollioides* and *P. delavayi*. *P. lutea* was positioned between these two groups:

P. potanini — *P. potanini* var. *trollioides*

P. potanini f. *alba* — *P. delavayi*

P. lutea

Taxonomic treatments of this complex in the past have been based on flower colour, numbers of bracts and sepals and lobe shape of leaves of each taxon. We observed these characteristics in each taxon in the field and concluded that the flower colour was useful in identifying the different taxa in the field even where several taxa grew together. There was substantial heterogeneity in lobe shape of leaves, number of bracts and sepals among five taxa examined indicating that these factors would not be particularly useful in discriminating between the taxa examined.

CONCLUSION

The karyotypic evidence presented in this paper conforms earlier findings suggesting taht taxa in the *P. delavayi* complex be closely related. The C-banding patterns differentiate the members of this complex.

However, the C-banding differentiations in this complex occur only in the C-banding numbers and positions at the short arms of the chromosomes. The C-banding patterns, in conjunction with the morphological characteristics, would also suggest that taxa in this complex are closely related and may have a common ancestor. DAN fingerprinting using RAPDs and RFLPs coupled with sequence data may be required to further unravel the relationships between the taxa in this complex and other members of the genus. Furthermore, additional studies are required to qualify the relationship between C-banding patterns and flower colour.

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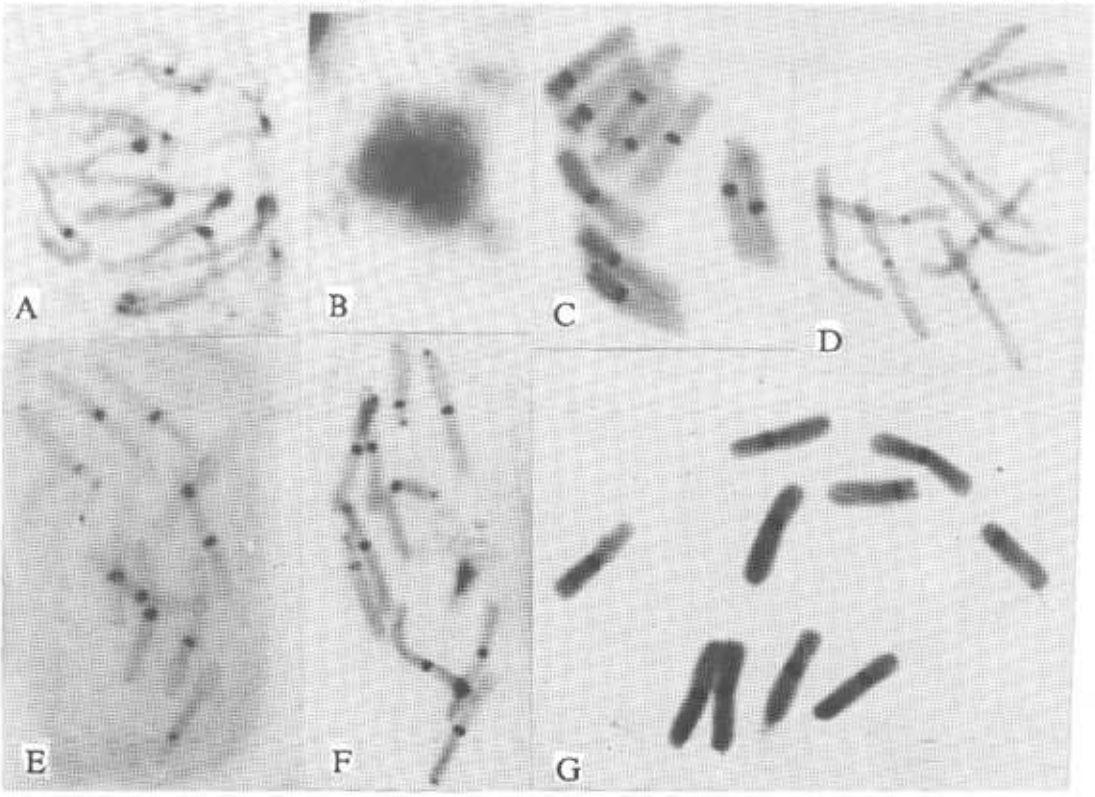
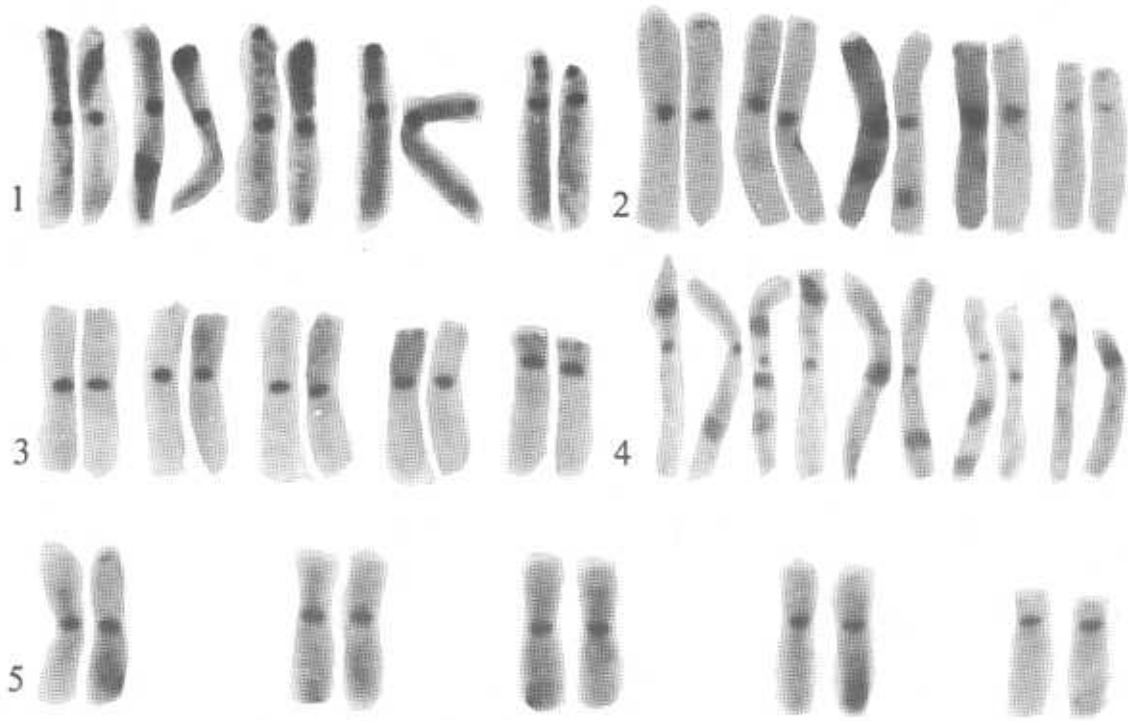
Explanations of Plate Ⅰ

1. Typical C-band patterns at prophase, interphase, metaphase of *P. delavayi* complex from Yunnan, China.

A. Prophase of *P. delavayi* Franchet; B. Interphase of *P. delavayi* Franchet; C. and 3. *P. lutea* Franchet ex Delavay; D. and 4. *P. potanini* var. *trollioides (Stapfex Stern) Stern; E. and 2. P. potanini* f. *alba* (Bean) Stern; F. and 1. *P. potanini* Komorov; G. and 5. *P. delavayi* Franchet.





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